þÿ D y n a m i c s o f a h o s t p a r a s i t o i d i n t e r a c t i o n c l a r i f i and DNA sequencing

Mutanen, Marko

2020-05

Mutanen , M , Ovaskainen , O , Várkonyi , G , Itämies , J , Prosser , S W J , Hebert , P D N þÿ & H a n s k i , I 2 0 2 0 , ' D y n a m i c s o f a h o s t p a r a s i t o i d i n t e r a c t i o n c l a r i f i e DNA sequencing ' , Ecology Letters , vol. 23 , no. 5 , pp. 851-859 . https://doi.org/10.1111/ele.13486

http://hdl.handle.net/10138/317021
https://doi.org/10.1111/ele.13486

ECOLOGY LETTERS

Ecology Letters, (2020) **23**: 851–859 doi: 10.1111/ele.13486

LETTER

Dynamics of a host–parasitoid interaction clarified by modelling and DNA sequencing

Marko Mutanen,[1]* Otso Ovaskainen,[2,3] Gergely Várkonyi,[4] Juhani Itämies,[5] Sean W. J. Prosser,[6] Paul D. N. Hebert[6] and Ilkka Hanski[7,†]

Abstract

It has been hypothesised that the 2-year oscillations in abundance of *Xestia* moths are mediated by interactions with 1-year *Ophion* parasitoid wasps. We tested this hypothesis by modelling a 35-year time series of *Xestia* and *Ophion* from Northern Finland. Additionally, we used DNA barcoding to ascertain the species diversity of *Ophion* and targeted amplicon sequencing of their gut contents to confirm their larval hosts. Modelling of the time-series data strongly supported the hypothesised host–parasitoid dynamics and that periodic occurrence of *Xestia* moths is mediated by *Ophion*. DNA barcodes revealed that *Ophion* included five species rather than just one while targeted amplicon sequencing verified that *Ophion* does parasitise *Xestia*. At least one *Ophion* species employs 1-year *Syngrapha interrogationis* as an alternate host, but it did not detectably affect *Xestia–Ophion* dynamics. We also demonstrate the previously unrecognised complexity of this system due to cryptic parasitoid diversity.

Keywords

DNA barcoding, MAPL, *Ophion*, periodic occurrence, population dynamics, *Xestia*.

Ecology Letters (2020) **23**: 851–859

INTRODUCTION

Determining what drives the dynamics of natural populations is difficult. A group of about 20 species of *Xestia* moths that are very abundant in the circumboreal region show an unusual demographic pattern. Their populations show alternate year oscillations in abundance with numbers being roughly two orders of magnitude greater in years when moths are abundant than when they are rare (Mikkola 1976; Mikkola & Kononenko 1989; Várkonyi *et al*. 2002; Kankare *et al*. 2002). As a particularly remarkable feature, multiple species share similar patterns of abundance and rarity. Rearing studies show that the development time in these species is invariably two years so there are two more or less independent cohorts at each locality, one hatching in odd years and the other in even years (Várkonyi *et al*. 2002). This raises a key question – what mechanism sustains the systematic, persistent abundance difference between the two coexisting but temporally isolated cohorts? Two main hypotheses have been proposed: competition between the cohorts (e.g., Bulmer 1977; Heliövaara & Väisänen 1986; Heliövaara *et al*. 1994) and interaction between the cohorts via a natural enemy with a 1-year life cycle, such as a parasitoid wasp, which by switching between the two host cohorts keeps the rare cohort rare (e.g., Mikkola 1976; Bulmer 1977; Heliövaara *et al*. 1994) (Figure S1). Empirical time series data for *Xestia* and a parasitoid wasp in the genus *Ophion* have provided strong indirect support for the host–parasitoid hypothesis (Rost *et al*. 2001; Várkonyi *et al*. 2002). A further feature of the dynamics is large-scale spatial variation in the identity of the common cohort. For instance, in Western Finnish Lapland the even year cohort is abundant, whereas in Eastern Lapland the odd year cohort is abundant, with a narrow transition zone (Mikkola 1976; Rost *et al*. 2001; Várkonyi 2003). Rost *et al*. (2001) showed that a spatial host–parasitoid model that incorporates environmental heterogeneity predicts spatio-temporal dynamics that are consistent with these empirical observations.

The *Ophion* species in the time series analysed by Várkonyi *et al*. (2002) was identified as *O. luteus* by GV and confirmed by J.P. Brock, whose revision of the genus (Brock 1982) was then the best resource for separating species in this genus which presents unusual taxonomic difficulty. Broad *et al*. (2015) elucidated the natural history of *O. luteus* in Britain and Germany. They found that it attacks late-instar larvae of moths in another noctuid genus, *Agrotis*, a result contrary to the assumption of the *Xestia–Ophion* hypothesis which requires both host specialization and the attack of early-instar host. However, it remains possible that the dominant autumnal *Ophion* species in Finnish Lapland is a separate cryptic species, morphologically close to *O. luteus*. If so, references to *O. luteus* in Rost *et al*. (2001), Kankare *et al*. (2002), Várkonyi *et al*. (2002) and Várkonyi (2003) would be

[1]*Ecology and Genetics Research Unit, University of Oulu, FI-90014, Finland Oulu,*
[2]*Organismal and Evolutionary Biology Research Programme, University of Helsinki, FI-00014, Finland Helsinki,*
[3]*Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, N-7491, Trondheim, Norway*
[4]*Friendship Park Research Centre, Finnish Environment Institute, FI-88900, Kuhmo, Finland*
[5]*Kaitoväylä 25 A 6, 90570, Oulu, Finland*
[6]*Centre for Biodiversity Genomics, University of Guelph, Guelph, ON, N1G 2W1, Canada*
[7]*Department of Biosciences, Metapopulation Research Center, University of Helsinki, Helsinki, Finland*
**Correspondence: E-mail: marko.mutanen@oulu.fi*
[†]*Deceased.*

incorrect, and the taxonomy of *Ophion* needs to be revisited. In fact, the taxonomy of Swedish *Ophion* have been recently revised (Johansson & Cederberg 2019), potentially providing new insights into the *Ophion* diversity in Finnish Lapland.

Unfortunately, extensive rearing studies have failed to prove that *Ophion* parasitise *Xestia* (Várkonyi *et al*. 2002). However, it has been shown, under controlled conditions, that host DNA can be retrieved from parasitoid wasps after metamorphosis using an approach termed Molecular Analysis of Parasitoid Linkages (MAPL) method (Rougerie *et al*. 2011), making it possible to directly ascertain the identity of their host species. Wirta *et al*. (2014) elucidated host–parasitoid interactions in an Arctic community by sequencing short, diagnostic segments of the DNA barcode region of the COI gene from the gut contents of adult parasitoids and compared these sequences with those of potential hosts.

Here, we aimed to provide new insights on *Xestia–Ophion* relationships and to rigorously test the underlying host–parasitoid hypothesis based on long time series collected for both hosts and parasitoids. First, we used MAPL to genetically ascertain the host species for *Ophion*. Second, we sequenced the barcode region of the *Ophion* wasps to ascertain if there was just a single or several cryptic species participating in the system, similar to the situation noted in other parasitoids wasps (Smith *et al*. 2008, 2013) and flies (Smith *et al*. 2006, 2007). Third, we modelled a 35-year long time series of *Ophion* and *Xestia* to test if these empirical data support the host–parasitoid hypothesis. Finally, we evaluated the impacts of potential alternative hosts for *Ophion* apart from *Xestia*. Várkonyi *et al*. (2002) analysed a 22-year time series (until 1999) from the same time series and found strong indirect support for the host–parasitoid hypothesis. By extending the time series by 13 years, we now test the generality of the previous result, and compare a set of alternative models to test for evidence of some other moths than *Xestia* acting as alternate hosts for *Ophion*.

We provide strong statistical support for the host–parasitoid hypothesis based on unusually long time series, demonstrate that the *Ophion* parasitoid community is a species complex, and provide, for the first time, direct genetic evidence of host use of *Ophion*.

MATERIAL AND METHODS

Long-term insect monitoring programme

Initiated in 1978, the Värriö insect monitoring programme was designed as a long-term assessment of the moth fauna at this subarctic site based on specimens collected by deploying 11 light traps. The trap transect is about 1300 m long, and ranges in altitude from 340 to 470 m a.s.l. (Pulliainen & Itämies 1988). Three traps are in an old-growth Scots pine (*Pinus sylvestris*) forest, three traps in a ravine of spruce-dominated (*Picea abies*) mixed forest, three traps in a mountain birch (*Betula pubescens* ssp. *czerepanovii*) forest on the northern slope of the Värriötunturi fell, and two traps on the treeless summit of the fell. Traps are similar to those described by Jalas (1975) and use 500-W blended light lamps which are illuminated from 20:00 to 08:00 from mid-May until mid-October. Samples were collected every morning, stored in a freezer, and moths were identified by JI. The voucher specimens are deposited in the Zoological Museum of the University of Oulu. Conveniently, the same traps also collected adult *Ophion* as they fly during the night and are strongly attracted to light.

Sampling of *Ophion* for DNA analyses

Ophion wasps have been separated from the rest of the trap samples since 1978 with dry specimens preserved in plastic jars. Material collected in 2012 was moved to 100% ethanol to slow DNA degradation. To clarify *Ophion* species diversity and their host species in the study area, we selected 190 specimens for barcode analysis. To analyse possible temporal variation in species composition, specimens from several years were examined with a focus on recent material to ensure high quality DNA. As a result, the specimens derived from 4 years: 2008 (50), 2009 (19), 2011 (31), and 2012 (90). One morphologically distinct specimen (later found to be *O. costatus*) was included for comparison.

The entire metasoma was used as a source of tissue for DNA extraction while the rest of the specimen was preserved as a voucher. To avoid contamination, each metasoma was separately washed in alcohol three times and subsequently cleaned of all visible adherent material, such as moth wing scales, with a brush under microscope. Tissue samples were placed in 96-well microplates for subsequent DNA extraction.

DNA-based characterisation of *Ophion* species diversity and their hosts

DNA was extracted from each metasoma by incubating it in lysis buffer (700 mM guanidine thiocyanate (Sigma), 30 mM EDTA pH 8.0 (Fisher Scientific), 30 mM Tris-HCl pH 8.0 (Sigma), 0.5% Triton X-100 (Sigma), 5% Tween-20 (Fluka Analytical), 2 mg/mL Proteinase K (Promega)) at 56 °C for 18 h. DNA was purified using the silica membrane-based method of Ivanova *et al*. (2006).

A 658 bp segment of the mitochondrial COI 5' terminus, i.e. the standard DNA barcode (Hebert *et al*. 2003), was sequenced at the Centre for Biodiversity Genomics in Guelph to assess species diversity in the *O. luteus* complex. The COI barcode region was amplified using primers LepF1 + LepR1, and, in cases where they did not yield an amplicon, two shorter, overlapping fragments were amplified using the primer pairs LepF1 + C_ANTMR1D and RonMWASPdeg_t1 + LepR1. All amplification reactions consisted of 2 μL of Hyclone ultrapure water (Thermo Scientific), 6.25 μL of 10% D-(+)-trehalose dihydrate (Fluka Analytical), 0.0625 μL of 10 mM dNTP (KAPA Biosystems), 1.25 μL of 10X PlatinumTaq buffer (Invitrogen), 0.625 μL of 50 mM $MgCl_2$ (Invitrogen), 0.125 μL of each primer, 0.060 μL of 5 U/μL PlatinumTaq DNA Polymerase (Invitrogen), and 2 μL of DNA for a total reaction volume of 12.5 μL. Sanger sequencing, each 96-plate with a negative control, was performed on an ABI 3730Xl sequencer using a modified BigDye protocol (Hajibabaei *et al*. 2005).

Since host DNA had been in the gut for several weeks, exposed to unfavorable conditions for DNA preservation, we

sought to amplify a short (148bp) fragment of the host COI gene. Fragments of this length are adequate for species identification, especially when a comprehensive reference library of potential target species is available (Hajibabaei *et al*. 2006; Mutanen *et al*. 2015). In the present case, all potential hosts of *Ophion* are Lepidoptera and there is a reference library for every lepidopteran species from the study region. Lepidoptera-specific primers, MAPL_LepF1_t1 and MAPL_LepR1_t1 (Wirta *et al*. 2014), were used which selectively amplify a 148 bp region of the COI barcode sequence which was then sequenced on an ABI 3730XL sequencer following Hajibabaei *et al*. (2005). Trace files were edited in CodonCode (CodonCode Corporation, Dedham, Massachusetts) and uploaded to BOLD as stated below.

A recent revision of the Swedish fauna of *Ophion* (Johansson & Cederberg 2019) was used to assign each specimen to a species.

All sequence and voucher data, including specimen collection data, high-resolution images of specimens, DNA sequences, GenBank accession numbers and raw trace files were deposited in BOLD (www.boldsystems.org) and all data are publicly available in the data set DS-OPGUT at dx.doi.org/10.5883/DS-OPGUT. The COI sequences were compared to those of Johansson & Cederberg (2019) for taxonomic identifications.

Host–parasitoid models

The Xestia–Ophion *model*

We use as our starting point the model described by Rost *et al*. (2001) and Várkonyi *et al*. (2002), given by

$$N_{t+2} = N_t g(N_t) f(P_t), \quad (1)$$
$$P_{t+1} = N_t(1 - f(P_t)), \quad (2)$$

where N_t and P_t denote population sizes of the host (*Xestia*) and parasitoid (*Ophion*), respectively. The function $g(N)$ describes the population growth rate of the host while the function $f(P)$ models the fraction of hosts that are not parasitised. These equations assume the host has a 2-year life cycle while the parasitoid has a 1-year life cycle. There are thus two independent host populations, one enclosing in even and the other in odd years. The model assumes no direct interaction between the two host cohorts, but they are coupled through the parasitoid.

We assume the Ricker model for host population dynamics and the model of Rost *et al*. (2001) for the parasitoid numerical response

$$g(N) = \exp\left[r\left(1 - \frac{N}{K}\right)\right], \quad (3)$$
$$f(P) = \frac{1 - e^{-aP}}{aP}. \quad (4)$$

This deterministic model has three parameters, which are assumed to be the same for the two *Xestia* cohorts: a is the search efficiency of the parasitoid, and r and K are, respectively, the growth rate and the carrying capacity of the host in the absence of the parasitoid. Rost *et al*. (2001) used the scaling $\tilde{N} = N/K$, $\tilde{P} = P/K$ and $\tilde{a} = aK$, in which case eqn (3) simplifies to $g(\tilde{N}) = \exp[r(1 - \tilde{N})]$, and eqn (4) to $f(\tilde{P}) = [1 - e^{-a\tilde{P}}]/a\tilde{P}$, and thus the model has only two parameters ($\tilde{a}$ and r). However, we proceed with the unscaled version of the model.

Model with an alternative host

Result from the MAPL analysis (see Results) suggested that *Ophion* might also parasitise *Syngrapha interrogationis*, a moth with a 1-year life cycle. In the absence of empirical data, we assume *Syngrapha* follows the same model as *Xestia,* but with a 1-year life cycle, and with different parameters. The model with the two host species (denoted by superscripts X and S for *Xestia* and *Syngrapha*, respectively) thus reads

$$N^X_{t+2} = N^X_t g^X(N^X_t) f^X(P_t), \quad (5)$$
$$N^S_{t+1} = N^S_t g^S(N^S_t) f^S(P_t), \quad (6)$$
$$P_{t+1} = N^X_t(1 - f^X(P_t)) + N^S_t(1 - f^S(P_t)) \quad (7)$$

and it has six parameters: a_X, a_S, K_X, K_S, r_X and r_S.

Fitting the model to empirical data

Data are available for the years 1978–2012, except that data for *Ophion* are missing for 2006 and 2010. As the abundance observations are based on 11 traps, they capture only a small proportion of the local populations. We $\log(x + 1)$ transformed the count data and assumed that observation error is normally distributed at this scale. We denote the error variance related to the observation process by σ^2, and assume it to be the same for the two hosts and the parasitoid.

We fitted the *Xestia–Ophion* model (eqns 1–2) and the *Xestia–Ophion–Syngrapha* model (eqns 5–7) to the data using Bayesian inference. We computed the likelihood of the data as the product of the likelihoods of the yearly transitions for the hosts and the parasitoid, all conditional on the observed values in previous years. In the *Xestia–Ophion* model, the estimated parameters are the process model parameters a, r, and K, and the observation model parameter σ. The *Xestia–Ophion–Syngrapha* model additionally has the parameters a, r, and K for *Syngrapha*. We assumed for all log-transformed parameters the essentially uninformative normal prior with mean 0 and standard deviation 10. The exception was $\log(\sigma)$, for which we set a normal prior with mean and standard deviation selected so that σ belonged to the interval from 0.5 to 1.0 with 95% prior credibility. These values were selected by examining the consistency among the counts in different traps which were pooled for the present analysis (see Appendix for details). We sampled the posterior with a random walk Metropolis–Hastings algorithm (programmed with Mathematica) with adaptive adjustment of the normal proposals during the initial 1,000 iterations. We ran the model for 20,000 iterations and discarded the initial half as a burn-in.

Testing the relative strength of interaction between *Syngrapha* and *Ophion*

We examined this question in two ways, by asking (1) whether accounting for *Ophion* helps to predict the dynamics of *Syngrapha*, and (2) whether accounting for *Syngrapha* helps to

predict the dynamics of *Ophion*. In this context, we consider the model given by eqns 5–7 as the full model, and the model

$$N^X_{t+2} = N^X_t g^X\left(N^X_t\right) f^X(P_t), \tag{8}$$

$$N^S_{t+1} = N^S_t g^S\left(N^S_t\right), \tag{9}$$

$$P_{t+1} = N^X_t\left(1 - f^X(P_t)\right) \tag{10}$$

as the reduced model that lacks the interaction between *Ophion* and *Syngrapha*. Note that the full model has one more parameter (a_S) than the reduced model.

To address question (1), we compared the full and reduced models by computing difference in the log-transformed maximum likelihood (ML) value related to the response variable N^S_t. To address question (2), we compared the full and reduced models by computing their difference for the log-transformed ML value related to the response variable P_t.

We computed the null distribution of the ML likelihood values in two ways. Null Distribution A was obtained by repeating the model fitting after replacing the *Syngrapha* time series by one of nine other control moth species which are common in the study area but are unlikely to be hosts for *Ophion*. Null Distribution B was obtained by repeating the model fitting for both *Syngrapha* and the other nine moth species, but now randomly permuting the time series, twice for each of the ten species (*Syngrapha* and the nine control moth species).

There are two reasons why we did not apply DIC-based model selection but derived the distribution of null values for the ML likelihood by approaches A and B. The first reason is that DIC (or other standard model selection tools) may deliver incorrect outcomes with multispecies time-series data that contain dependencies generated by the dynamics. The second reason is that comparison between Null Distributions A and B makes it possible to disentangle cases of complete noise (data vs. Null Distribution B) and an apparent correlation between *Syngrapha* and *Ophion* that could be generated, for example, by covariation with some environmental feature that influences all moth species (data vs. Null Distribution A).

RESULTS

Xestia and *Ophion* dynamics

Over the 35 years, the light traps sampled 15 682 individuals of eight species of *Xestia*. Two were dominant; *X. tecta* (9396 individuals) and *X. alpicola* (4600) accounted for 89% of all individuals. Table S1 in the Supplementary Material reports the number of each species collected in each year. In the same period, 8,932 individuals of *Ophion* were caught excluding 2006 and 2010 where the samples were lost.

Figure 1 shows the time series of log-transformed total abundances for the eight species of *Xestia* (Figure 1a), *Ophion* (Figure 1b) and *Syngrapha interrogationis* (Figure 1c). The two-year periodicity is striking in *Xestia* with the odd-year cohort being far more abundant without exception than the even-year cohort for all 35 years (Figure 1a). Similar periodicity is not seen in *S. interrogationis* which has a 1-year life cycle. The dynamics of *Ophion* show a similar pattern to *Xestia*, though now we have a single population with a true 2-year cycle, with population size greater in the even- than odd-years (Figure 1b). The dynamics of *Ophion* are somewhat less regular than those of *Xestia*, and we describe a plausible biological explanation for this difference (see Discussion).

The time series suggests a longer cyclic component with a roughly 15-year cycle period (Figure 1d), a pattern already noted by Várkonyi *et al.* (2002) and now strengthened by the current longer time series.

Species diversity of *Ophion*

Previous morphological studies (Rost *et al.* 2001; Várkonyi *et al.* 2002) assumed the presence of just a single species of *Ophion (O. luteus)* in the study area. We tested this assumption based on the COI sequences obtained from 180 of the 190 individuals that were analysed. Maximum p-distance divergence within the 180 *Ophion* was 12.58%. Sequences for the *O. luteus* complex formed five distinct clusters with the morphologically distinct *O. costatus* comprising a sixth group (Figure 2). Maximum p-distance divergences within each cluster ranged from 0% to 0.46%, while the minimum divergences between the clusters varied from 1.96% to 12.19% (Figure 2). Based on Johansson & Cederberg (2019), most (69%) of the specimens of *Ophion* actually represent *O. kevoensis* Jussila, 1965 (cluster #1), while 26% represent *O. inclinans* Johansson, 2019 (cluster #3). *Ophion broadi* Johansson, 2019 (cluster #2), *Ophion* sp. (cluster #4) and *O. tenuicornis* Johansson, 2019 (cluster #5) were uncommon, being represented by two, one, and five individuals, respectively (Figure S2).

The relative abundance of the two dominant *Ophion* species (*O. kevoensis* and *O. inclinans*) was not random with respect to odd and even years. Just four individuals of *O. kevoensis* were collected in odd years versus 120 in even years. By contrast, 34 individuals of *O. inclinans* were collected in odd years versus 13 in even years. Considering all five species, the difference between the years is highly significant for *O. kevoensis* ($\chi^2 = 69.5$, $P < 0.0001$), whereas in *O. inclinans* the difference is only marginally so ($\chi^2 = 4.6$, $P = 0.03$).

Host species revealed by MAPL

Gut contents of 50 *Ophion* individuals produced a lepidopteran sequence that derived from 11 different species. Many (35) of these cases involved taxa that are very improbable hosts, suggesting contamination. Fifteen cases involved species that are too small to be hosts (Tortricidae: 4 species, 12 observations; Gelechiidae: 1 species, 1 observation, Geometridae: 2 species, 2 observations). Further 20 cases involved the noctuid *Lithomoia solidaginis* that is an unlikely host due to its life history. *Ophion* lay their eggs in the larvae of Lepidoptera (e.g., Quicke 2015), while *Lithomoia* overwinters as an egg (Mikkola & Jalas 1977) so its larvae are unavailable for the autumnal *Ophion* at Värriö.

The recovery of these sequences might reflect *Ophion* contaminated by other insects simultaneously attracted by the light trap. To test this possibility, we examined if the spurious hosts occurred in the same trap night as the *Ophion* specimen. For *L. solidaginis*, this was true in all 20 cases. Among the

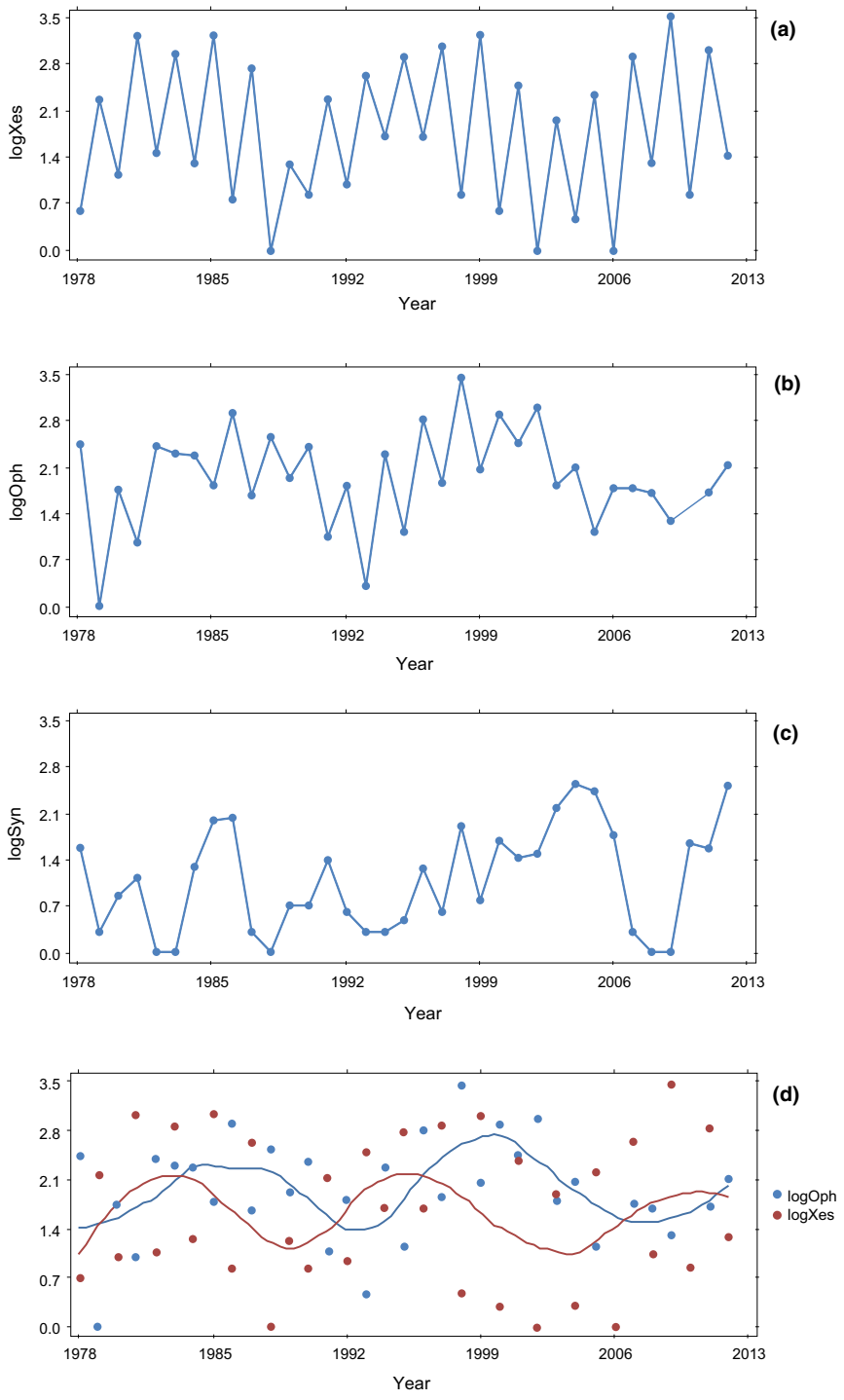

Figure 1 Time series of *Xestia* (a), *Ophion* (b), *Syngrapha interrogationis* (c) and the smoothed time series for *Xestia* + *Ophion* (d).

170 cases where the *Ophion* gut content did not produce a *L. solidaginis* sequence, *L. solidaginis* was recorded in the same sample with an *Ophion* specimen in 101 cases. The 121 cases with co-occurrence could be distributed among the 190 *Ophion* samples in $n = 190!/(121! \times 69!)$ ways. Out of these, $m = 170!/(101! \times 69!)$ are such for which the 20 co-occurrences would have been found for those cases where the *Ophion* gut content produced *L. solidaginis*. Because the probability of obtaining this result by chance is $m/n \approx 0.00006$, we consider it likely that the *L. solidaginis* host records reflect contamination of *Ophion* adults by other specimens in the trap.

The remaining 15 records represented three species, *Syngrapha interrogationis* (5), *Xestia alpicola* (5) and *X. speciosa* (5), which are all potential hosts based on their size and life history – although *S. interrogationis*, unlike the two *Xestia*

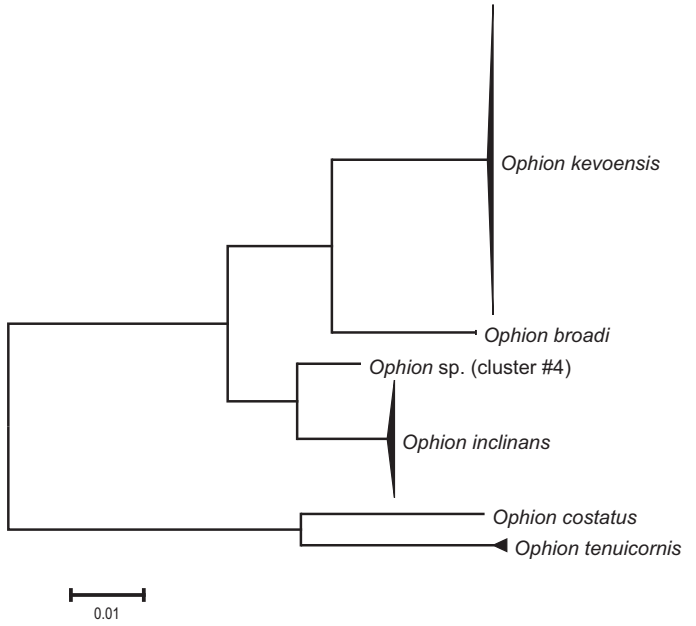

Figure 2 Neighbour-Joining (p-distance) tree for the *Ophion*. Height of the triangle is proportional to the sampling intensity, and its depth to the genetic variability within the species.

species – has a 1-yr life cycle so it is extremely unlikely to contribute to the alternate-year dynamics of *Ophion*. All of these records derived from 2012. Among the 15 likely host records, 14 derived from *Ophion kevoensis* and one from *Ophion inclinans* (See Figure S2 for *Ophion* specimens with host confirmed). In the case of *X. alpicola* and *X. speciosa*, the host and *Ophion* never occurred together in a trap catch. In fact, no specimens of *X. alpicola* and only a single *X. speciosa* were captured during 2012. In the case of *S. interrogationis*, the host and *Ophion* occurred in the same trap in the same night in three of five cases. However, the first host records (13 August 2012) were made before the first moth was caught (16 August 2012) suggesting that *S. interrogationis* is likely a host.

Modelling results

Table 1 provides the parameter estimates for the *Xestia–Ophion* model. Parameters r and a are positively correlated ($r = 0.42$) in the posterior distribution, whereas other parameter pairs show no strong posterior correlations (maximum absolute correlation 0.06). The dynamics of the two-species model are determined by r_X and the parameter combination $a_X K_X$ (Rost *et al.* 2001), of which the latter could not be estimated accurately (Table 1). Between these parameters, there is more uncertainty in the estimate of the host carrying capacity K_X than for parasitoid searching efficiency a_X.

Figure 3 shows the bifurcation diagram for *Xestia* and *Ophion* dynamics in the *Xestia–Ophion* model. We assume here the estimated values $r_X = 2.5$ and $K_X = 2800$ (Table 1), and vary the value of the parasitoid searching efficiency a_X (on the horizontal axis). The diagram shows six distinct phases (Roman numerals I–VI in Figure 3). The dynamics representative of each phase are illustrated in Figure S3. For

Table 1 Parameter estimates of the *Xestia–Ophion* model (*X–O*; eqns 1–2), the full *Xestia-Ophion–Syngrapha* model (*X–O–S* full; eqns 5–7), and the reduced *Xestia–Ophion– Syngrapha* model (*X–O–S* reduced; eqns 8–10). All values show posterior median estimate (95% credibility interval)

Model/ parameter	*X–O*	*X–O–S* full	*X–O–S* reduced
r_X	2.5 (2.1…4.9)	2.5 (2.1…4.6)	2.5 (2.0…4.9)
a_X	0.12 (0.08…1.40)	0.11 (0.07…0.93)	0.12 (0.08…1.21)
K_X	2800 (2000…1.6 · 10^6)	2500 (1900…6.7 · 10^5)	2700 (2000…2.1 · 10^7)
$a_X K_X$	410 (190…2.7 · 10^5)	310 (170…1.1 · 10^5)	400 (190…3.4 · 10^6)
σ	1.41 (1.35…1.66)	1.29 (1.24…1.45)	1.44 (1.39…1.62)
r_S	NA	0.004 (3 · 10^{-5}…0.91)	2·10^{-4}(4 · 10^{-6}…0.26)
a_S	NA	0.003 (0.002…0.009)	NA
K_S	NA	210 (17…2.8 · 10^9)	32 (1…1.6 · 10^9)

very small values of a_X (Phase I), the parasitoid is at a stable equilibrium at a very low level and the host shows a two-point cycle, generated by its intrinsic dynamics (note that r_X is greater than 2), which are apparently synchronised by the interaction with the parasitoid. Somewhat greater values of a_X (Phase II) lead to stable dynamics, with equally large odd-year and even-year cohorts. For still larger values of a_X (Phase III), the dynamics become cyclic, but without a 2-year component. For still larger values of a_X (Phase IV), the dynamics turn into a 4-point cycle, with 2 peak-year and 2 low-year densities, hence essentially representing a clear abundance difference between the two cohorts. Still larger values a_X (Phase V), such as the posterior median estimate of $a_X = 0.12$, lead to more complex dynamics, which involve two main components, the alteration between the peak and low years, and a longer cyclic component, roughly 15 years in length. Finally, in Phase VI the dynamics are similar to Phase I, with the host showing a 2-point cycle and the parasitoid stable dynamics at very low density. Figure 4 shows the predicted dynamics for the posterior median parameter values. The dynamics are qualitatively similar to the observed ones (Figure 1). Interestingly, the model replicates both the 2-year cyclic component (Figure 4ac), and the longer-term (ca. 15 years) cyclic component (Figure 4bd).

The estimates of the shared parameters are similar for the *Xestia–Ophion* model and the *Xestia–Ophion–Syngrapha* full model, suggesting that the presence of *Syngrapha* does not greatly affect the dynamics of *Ophion* and *Xestia*. Both the growth rate r_S and the carrying capacity K_S remained poorly estimated. The full model yielded higher log-likelihood values than the reduced model, the difference being ca. 5 units for the response variable N_t^S, ca. 10 units for the response variable P_t, and ca 2 units for the response variable N_t^X (Figure 5). However, the observed values are not distinct from null distributions, A and B, which moreover did not differ substantially from each other. Thus, the time-series data do not provide evidence for *Syngrapha* being an ecologically important alternative host for *Ophion* in the sense that their dynamics would be strongly coupled.

DISCUSSION

The statistical modelling of the time-series data provided very strong evidence for a host–parasitoid interaction between

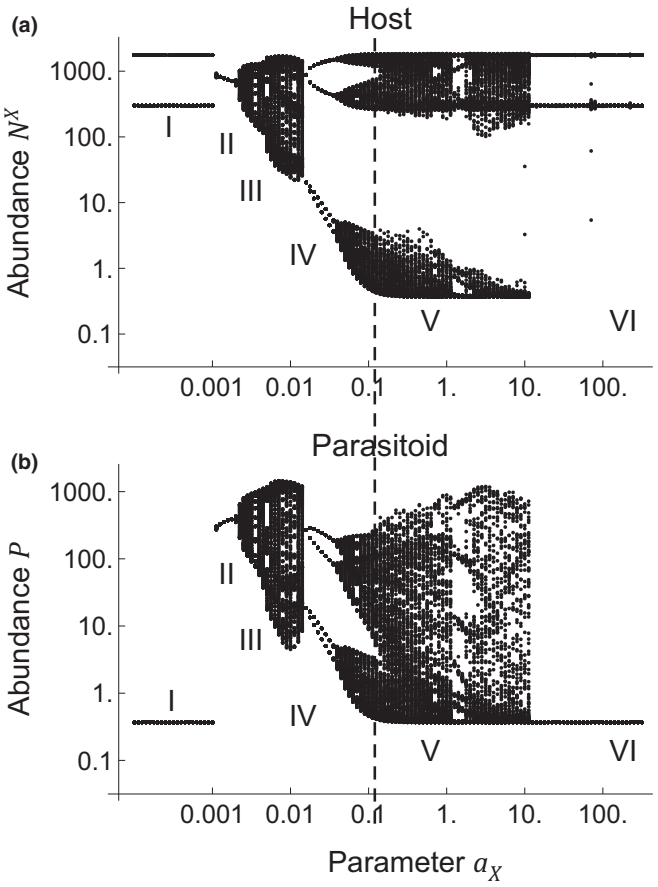

Figure 3 A bifurcation diagram of the *Xestia–Ophion* model. Panel a shows the host *Xestia* (eqn 1) while panel b shows the parasitoid *Ophion* (eqn. 2). We assumed the posterior median parameter values for the carrying capacity $K = 2800$ and growth rate $r = 2.5$, and varied search efficiency parameter a. For each parameter value, we simulated the dynamics for 500 years, out of which the first 200 were ignored as a transient. The dashed vertical line shows the posterior median estimate of the parameter α_x. The Roman numerals I-VI refer to different phases of the bifurcation diagram. Note the logarithmic scales in both axes.

Xestia and *Ophion*. The host–parasitoid model, when fitted to the data, replicated the very regular 2-year periodic behaviour of the host (Figure 1a vs. Figure 4a), and the somewhat less regular 2-year cyclic behaviour of the parasitoid (Figure 1b vs. Figure 4c), as well as the longer cyclic component apparent in the empirical data (Figure 1d vs. Figure 4b,d). By contrast, modelling did not give additional support for *Syngrapha* being an alternative host of *Ophion,* as including *Syngrapha* in the model did not improve the likelihood of observing the data more than expected by chance. One novelty of our statistical methodology is that we quantified the level of likelihood increase expected by chance under two ecologically meaningful null hypotheses, in which we either permuted *Syngrapha* data over the years, or replaced *Syngrapha* data with data from other species for which there was no molecular or other evidence for their role as potential hosts. As both types of null distributions generate the same result, our conclusion of the data not supporting *Syngrapha* as a dynamically important alternative host can be considered robust.

Our study demonstrates that DNA barcode reference libraries coupled with molecular techniques enable the dissection of species interactions in an unprecedented way. The present study benefitted from the fact that reference barcodes were available for all species of Lepidoptera known from Finland. Our study is among the first to apply the MAPL approach (Rougerie *et al*. 2011) to clarify host–parasitoid relationships, and we did so to resolve an enigma that had eluded resolution despite years of fieldwork and many rearing and laboratory experiments. The reasons for earlier failures remain unclear, but the possibilities include the (1) low probability of finding larvae of the rare cohort where the percent parasitism is presumed to be high and conversely the presumed very low parasitism rate in the common cohort, (2) changes in the behaviour of parasitised larvae which reduce their chances of discovery, or (3) high winter mortality of parasitised larvae under laboratory conditions. Since high-throughput sequencing permits the simultaneous acquisition of large amounts of data from many specimens, such approaches will soon be widely used to probe species interactions. A huge advantage of molecular methods is their capacity to simultaneously determine both host and parasitoid species from the same specimen. Since parasitoids ordinarily cause the death of their host, such data were previously difficult to obtain.

Our DNA barcode analysis of 180 *Ophion* sp. cf. *O. luteus* revealed five lineages showing deep sequence divergences. Although deep intraspecific divergences in mtDNA may result from other mechanisms, such as incomplete lineage sorting or *Wolbachia*-mediated spread of a distinct mtDNA sequence in a population through introgression (Funk & Omland 2003; Hurst & Jiggins 2005), we initially expected these lineages to represent five distinct but cryptic species. Three observations supported this conclusion. First, variation between the clusters is very high (14%) while intracluster is less than 0.5% in each case. Clusters are separated from each other by > 2% divergence with the mean divergence between closest clusters being 4.6%. Second, the five clusters were widely scattered among the many known species of the *Ophion* in a Neighbour-Joining tree with all data accessible in BOLD. Such phylogenetically deep polyphyly in mtDNA is rare in DNA barcode data; most reported cases likely represent cryptic diversity (Mutanen *et al*. 2016). Thirdly, the *Ophion* clusters showed non-random occurrence patterns in odd and even years. Furthermore, our data suggest that these taxa parasitise different hosts. During the review of this manuscript, a taxonomic revision of Swedish *Ophion* was published (Johansson & Cederberg 2019), enabling us to assign four of our five clusters to a named species. Both the host observations and host–parasitoid dynamics suggest that *O. kevoensis* is the main, or perhaps the only, parasitoid of periodic *Xestia* moths in the region. Perhaps the other four *Ophion* species occasionally parasitise *Xestia*, but *O. inclinans* must use a different species of Lepidoptera as its host. The presence of multiple species means that our modelling of *Xestia–Ophion* dynamics is based on empirical time series that contain noise because some of the *Ophion* specimens likely belong to species that do not parasitize *Xestia*. However, this weakness was not strong enough to blur resolution of the 2-year dynamics in the *Xestia–Ophion* system.

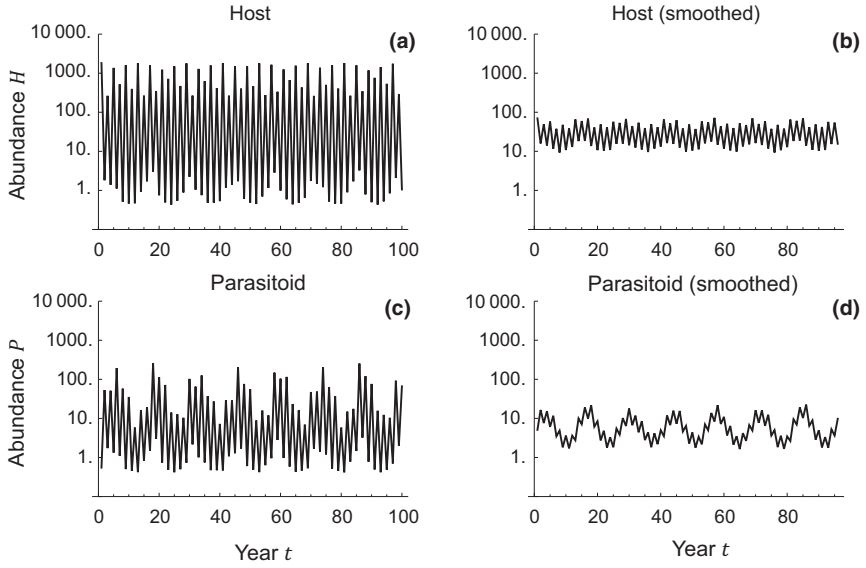

Figure 4 The predicted dynamics of the *Xestia–Ophion* model for posterior median parameter values ($K = 2800$, $r = 2.5$, $a = 0.12$). The left-hand panels (a and c) show the yearly dynamics of the host and parasitoid, while the right-hand panels (b and d) show the same data smoothed by a 5-year moving average.

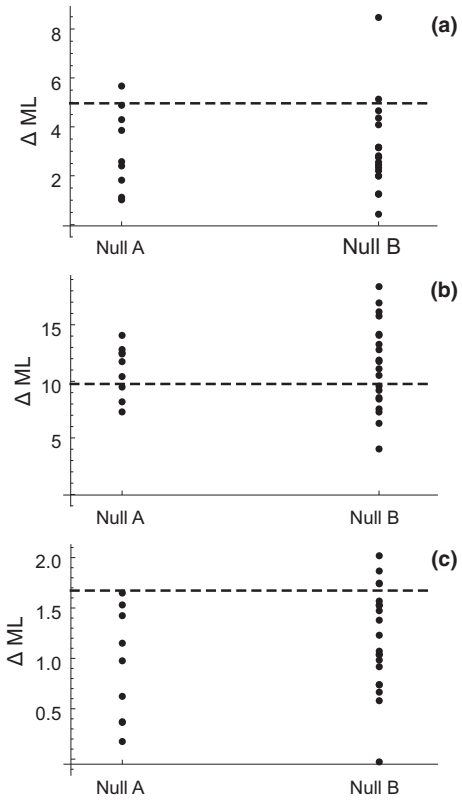

Figure 5 Statistical tests examining whether the time-series data support the alternative host hypothesis for *Syngrapha*. The difference in log-transformed maximal likelihood between the full and reduced models (ML). The panels correspond to the likelihoods computed for the response variables N_t^S (a), P_t (b), and N_t^X (c). The dashed line shows the value for the data, and the dots show the Null Distributions A and B (see text).

In conclusion, by coupling DNA-based approaches with long time series of both hosts and parasitoids, we obtained strong evidence that host–parasitoid interactions underpin the striking periodic occurrence of *Xestia*. We demonstrated that *Ophion* parasitoids have a crucial role in maintaining this system and showed that seemingly simple biological systems may be unexpectedly complex once cryptic diversity is evaluated. Furthermore, we showcased how DNA-based methods can reveal food webs in unprecedented detail. For future directions and further elucidating the *Xestia–Ophion* system, we propose (1) reducing incidence of false positives in the dietary analyses by collecting parasitoids in a way that minimises contact with other species and using disinfectant techniques to remove external DNA and/or dissecting the gut tract; (2) examining a large number of *Ophion* specimens with high-throughput technologies to advance understanding of their diversity and distribution; and (3) extending the investigations by Várkonyi *et al.* (2002) and Várkonyi (2003) whether other natural enemies of *Xestia* participate in the striking periodic dynamics of *Xestia* and *Ophion*.

ACKNOWLEDGEMENTS

This study was enabled by support from the Academy of Finland (Grant 284601 to IH, Grant 309581 to OO and Grant 277984 to MM), the Jane and Aatos Erkko Foundation (Grant to OO), and the Research Council of Norway through its Centres of Excellence Funding Scheme (223257) to OO via the Centre for Biodiversity Dynamics. Sequence analyses were largely covered by a Discovery grant to PDNH from NSERC. We are grateful to Niklas Johansson (Habo, Sweden) for taxonomic help with *Ophion* and Bess Hardwick (Helsinki, Finland) and Pirjo Appelgren (Kuhmo, Finland) for their assistance with the figures. Similarly, we are grateful to the staff of Värriö research station as well as Arja Itämies and Päivi Tanner and for taking care of insect collection and pre-sampling of specimens. Finally, we want to express our gratitude to three anonymous reviewers for useful comments that significantly improved the quality of our paper.

AUTHORSHIP

MM and IH designed the study. MM and GV designed and conducted the specimen sampling. JI identified moths from the Värriö monitoring programme. JI and GV separated *Ophion* wasps. OO and IH formulated the statistical time-series models and OO fitted them to the data and performed the analyses. SP and PH conducted DNA sequencing while MM and SP performed the sequence analyses. MM, IH and OO drafted the manuscript and all other authors aided in its revision.

DATA AVAILABILITY STATEMENT

All specimen and sequence data are publicly available through the BOLD dataset DS-OPGUT at dx.doi.org/10.5883/DS-OPGUT and from the Dryad Digital Repository: https://doi.org/10.5061/dryad.nzs7h44nh.

REFERENCES

Broad, G.R., Schnee, H. & Shaw, M.R. (2015). The hosts of *Ophion luteus* (Linnaeus) (Hymenoptera, Ichneumonidae, Ophioninae) in Europe. *J. Hymenopt. Res.*, 46, 115–125.

Brock, J.P. (1982). A systematic study of the genus *Ophion* in Britain (Hymenoptera, Ichneumonidae). *Tijdschr. Entomol.*, 125, 57–97.

Bulmer, M.G. (1977). Periodical insects. *American Naturalist*, 111, 1099–1117.

Funk, D.J. & Omland, K.E. (2003). Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annu. Rev. Ecol. Evol. Syst.*, 34, 397–423.

Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, R., Dooh, R.T., Kirk, S.L. *et al.* (2005). Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 360, 1959–1967.

Hajibabaei, M., Smith, M.A., Janzen, D.H., Rodriguez, J.J., Whitfield, J.B. & Hebert, P.D.N. (2006). A minimalist barcode can identify a specimen whose DNA is degraded. *Mol. Ecol. Notes*, 6, 959–964.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proc R. Soc. Lond. B.*, 270, 313–321.

Heliövaara, K. & Väisänen, R. (1986). Bugs in bags: intraspecific competition affects the biogeography of the alternate-year populations of *Aradus cinnamomius* (Heteroptera). *Oikos*, 47, 327–334.

Heliövaara, K., Väisänen, R. & Simon, C. (1994). Evolutionary ecology of periodical insects. *Trends Ecol. Evol.*, 9, 475–480.

Hurst, G.D.D. & Jiggins, F.M. (2005). Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proc. R. Soc. B.*, 272, 1525–1534.

Ivanova, N.V., deWaard, J.R. & Hebert, P.D.N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Mol. Ecol. Notes*, 6, 998–1002.

Jalas, I. (1975). Perhostenkeräilijän opas. Keuruu, p. 256.

Johansson, N. & Cederberg, B. (2019). Review of the Swedish species of *Ophion* (Hymenoptera: Ichneumonidae: Ophioninae) with the description of 17 new species and an illustrated key to Swedish species. *European Journal of Taxonomy*, 550, 1–136.

Kankare, M., Várkonyi, G. & Saccheri, I. (2002). Genetic differentiation between alternate-year cohorts of *Xestia tecta* (Lepidoptera, Noctuidae) in Finnish Lapland. *Hereditas*, 136, 169–176.

Mikkola, K. (1976). Alternate-year flight of northern *Xestia* species (Lep., Noctuidae) and its adaptive significance. *Ann. Entomol. Fenn.*, 42, 191–199.

Mikkola, K. & Jalas, I. (1977). *Suomen Perhoset. Yökköset*, Vol. 1. Helsinki, p. 256.

Mikkola, K. & Kononenko, V.S. (1989). Flight year of the alternate-year *Xestia* moths (Lepidoptera, Noctuidae) in north-eastern Siberia – A character from the Ice Ages? *Nota lepidop.*, 12, 144–152.

Mutanen, M., Kekkonen, M., Prosser, S.W.J., Hebert, P.D.N. & Kaila, L. (2015). One species in eight: DNA barcodes from type specimens resolve a taxonomic quagmire. *Mol. Ecol. Resour.*, 15, 967–984.

Mutanen, M., Kivelä, S.M., Vos, R.A., Doorenweerd, C., Ratnasingham, S., Hausmann, A. *et al.* (2016). Species-level para- and polyphyly in DNA barcode gene trees: Strong operational bias in European Lepidoptera. *Syst. Biol.*, 65, 1024–1040.

Pulliainen, E. & Itämies, J. (1988). *Xestia* communities (Lepidoptera, Noctuidae) in eastern Finnish Forest Lapland as indicated by light trapping sampling. *Holarctic Ecol.*, 11, 235–240.

Quicke, D.L.J. (2015). *The braconid and ichneumonid parasitoid wasps. Biology, systematics, evolution and ecology*. John Wiley & Sons, Chichester, UK, p. 681.

Rost, M., Várkonyi, G. & Hanski, I. (2001). Patterns of 2-year population cycles in spatially extended host–parasitoid systems. *Theor. Popul. Biol.*, 59, 223–233.

Rougerie, R., Smith, M.A., Fernandez-Triana, J.L., Lopez-Vaamonde, C., Ratnasingham, S. & Hebert, P.D.N. (2011). Molecular analysis of parasitoid linkages (MAPL): gut contents of adult parasitoid wasps reveal larval host. *Mol. Ecol.*, 20, 179–186.

Smith, M.A., Woodley, N., Hallwachs, W., Janzen, D.H. & Hebert, P.D.N. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proc. Natl Acad. Sci. USA*, 103, 3657–3662.

Smith, M.A., Wood, D.M., Janzen, D., Hallwachs, W. & Hebert, P.D.N. (2007). DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proc. Natl Acad. Sci. USA*, 104, 4967–4972.

Smith, M.A., Rodriguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Hallwachs, W. *et al.* (2008). Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proc. Natl Acad. Sci. USA*, 105, 12359–10.1073/pnas.0805319105.

Smith, M.A., Fernández-Triana, J.L., Eveleigh, E., Gómez, J., Guclu, C., Hallwachs, W. *et al.* (2013). DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after eight years and nearly 20,000 sequences. *Mol. Ecol. Resour.*, 13, 168–76.

Várkonyi, G. (2003). Population biology of periodic *Xestia* moths. Academic dissertation. *University of Helsinki.*, 100, pp. http://urn.fi/URN:ISBN:952-10-1319-2.

Várkonyi, G., Hanski, I., Rost, M., & Itämies, J., (2002). Host-parasitoid dynamics in periodic boreal moths. *Oikos*, 98, 421–430.

Wirta, H., Hebert, P.D.N., Kaartinen, R., Prosser, S.W., Várkonyi, G. & Roslin, T. (2014). Complementary molecular information changes our perception of food web structure. *Proc. Natl Acad. Sci. USA*, 111, 1885–1890.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Frederick Adler
Manuscript received 5 September 2019
First decision made 14 October 2019
Second decision made 29 January 2020
Manuscript accepted 14 February 2020